Adaptive evolution of a lactose-consuming *Saccharomyces cerevisiae* recombinant

P. M. R. Guimarães[1], J. François[2], J. L. Parrou[2], J. A. Teixeira[1] and L. Domingues[1]

[1] IBB – Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Portugal
[2] Laboratoire d'Ingenierie des Sistèmes Biologiques et Procédés, France

The construction of *Saccharomyces cerevisiae* strains with the ability to efficiently ferment lactose has biotechnological interest, particularly for the alcoholic fermentation of cheese whey (a high pollutant by-product of dairy industries). A flocculent lactose-consuming *S. cerevisiae* recombinant expressing the *LAC12* (lactose permease) and *LAC4* (beta-galactosidase) genes of *Kluyveromyces lactis* was previously constructed, but presented poor efficiency in the fermentation of lactose. Thus, it was subjected to a long-term adaptation experiment (serial transfer/dilution in lactose media), which yielded an evolved recombinant strain with strongly improved lactose fermentation phenotype. The lactose (25 g/L) fermentation parameters of the evolved strain were similar to *K. lactis* wild-type strain CBS2359. The evolved recombinant showed increased beta-galactosidase activity (>20-fold) and also improved lactose uptake rates, compared to the original recombinant. We found a 1593 bp deletion in the intergenic region between *LAC4* and *LAC12* (which works as common promoter region for these genes) in the plasmid isolated from the evolved recombinant. The results strongly suggest that the intact promoter does not mediate activation of transcription in the original recombinant, whereas the deletion triggered transcriptional activation of both the *LAC* genes in the evolved strain. We also found evidence that plasmid copy number is lower (about 10-fold difference) in the evolved strain compared to the original recombinant. We suggest that tuning of the heterologous *LAC* genes expression in the evolved recombinant was accomplished by interplay between decreased copy number of both genes, as consequence of decreased plasmid copy number, and different levels of transcriptional induction for *LAC4* and *LAC12*, resulting from the changed promoter structure. This study illustrates the usefulness of simple evolutionary engineering approaches in the improvement of genetically engineered strains that display poor efficiency. The evolved strain obtained displays a stable lactose fermentation phenotype and constitutes an attractive alternative for the fermentation of lactose-based media. This strain was able to completely ferment 140 g/L lactose (mineral medium, batch) yielding 63 g/L of ethanol. It also fermented completely cheese whey powder solution containing 140 g/L of lactose, producing 55 g/L of ethanol.